510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:
k043556

B. Purpose for Submission:
New product

C. Measurand:
Benzodiazepine

D. Type of Test:
Qualitative Immunoassay

E. Applicant:
Beckman Coulter Inc.

F. Proprietary and Established Names:
SYNCHRON Systems Benzodiazepines (BNZG) Reagent

G. Regulatory Information:

1. Regulation section:
   21 CFR §862.3170, Benzodiazepine test system
2. Classification:
   Class II
3. Product code:
   JXM
4. Panel:
   Toxicology (91)

H. Intended Use:

1. Intended use(s):
   This device is used in the diagnosis and treatment of benzodiazepine use or overdose.
2. Indication(s) for use:
   "BNZG reagent, when used in conjunction with SYNCHRON® System(s) and SYNCHRON® Systems Drugs of Abuse Testing (DAT) Urine Calibrators, is intended for the qualitative determination of Benzodiazepine (BNZG) in human urine at a cutoff of 200 ng/mL (oxazepam).

   The BNZG assay provides a rapid screening procedure for determining the presence of the analyte in urine. This test provides only a preliminary analytical result; a positive result by these assays should be confirmed by another generally accepted non-immunological method such as thin layer chromatography (TLC), gas chromatography (GC), or gas chromatography/mass spectrometry (GC/MS).

GC/MS is the preferred confirmatory method.

Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used."

3. Special conditions for use statement(s):
   This product is for prescription use only.
4. Special instrument requirements:
   Beckman Coulter Synchron LX and CX System families

I. Device Description:

The device consists of a single reagent cartridge divided into three chambers containing: polyclonal goat anti-benzodiazepine antibodies, glucose-6-phosphate, and NAD in buffer: glucose-6-dehydrogenase (G6PDH) labeled with benzodiazepine analog derivative in buffer: and β-glucuronidase derived from *E. coli*.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   SYNCHRON Systems BENZ Reagent
2. Predicate 510(k) number(s):
   k023048 and k944076
3. Comparison with predicate:
   The device and its predicate have the same intended use, stable liquid reagent, stability, calibrators, and controls. The BNZG reagent differs from the predicate in: the antibodies used, the use of glucuronidase pre-treatment, cross-reactivity, and the reagent volume used.

K. Standard/Guidance Document Referenced (if applicable):

Area of Study	Reference Procedure	Procedure Title
Precision	NCCLS EP5-A	User Evaluation of Precision Performance of Clinical Chemistry Devices
Interferences/ Cross-Reactivity	NCCLS EP7-A	Interference Testing in Clinical Chemistry

L. Test Principle:

This assay modifies the predicate assay by including a sample incubation step where glucuronide metabolites of benzodiazepines are hydrolyzed with β-glucuronidase. Liberated benzodiazepines are then incubated with a mixture of antibodies that can detect most benzodiazepines and glucose-6-phosphate dehydrogenase (G6PDH) conjugated benzodiazepine. The conjugate competes with free drug from the urine sample for a limited number of antibody binding sites. If no drug is present in the urine sample, the conjugate binds completely to the antibody and inhibits the activity of the enzyme. Thus there is a direct relationship between the presence of the drug and conversion of NAD substrate to NADH by the G6PDH. This results in an absorbance change that can be measured spectrophotometrically at 340 nm. A qualitative result is reported based on a comparison of the sample rate to the

calibrated (oxazepam) cutoff rate.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Imprecision studies were based on NCCLS Guideline EP5-A. Two levels of control material were used in a total of two assays per day, two replicates per assay, over 20 days.

Within-Run Imprecision of BNZG Reagent on Synchron CX and LX

Synchron CX (n=80)				***Synchron LX (n=80)***			
Sample	**Mean Rate (mA/min)**	**Std Dev**	**% C.V.**	**Sample**	**Mean Rate (mA/min)**	**Std Dev**	**% C.V.**
Control 1	409	2.1	0.5	Control 1	439	3.3	0.8
Control 2	447	3.4	0.8	Control 2	480	4.6	1.0
Pool	439	3.4	0.8	Pool	470	4.1	0.9

All samples urine based: Control 1 = 150 ng/mL Oxazepam, Control 2 = 300 ng/mL Oxazepam: Pool = 1250 ng/mL lorazepam glucuronide

Total Imprecision of BNZG Reagent on Synchron CX and LX

Synchron CX (n=80)				***Synchron LX (n=80)***			
Sample	**Mean Rate (mA/min)**	**Std Dev**	**% C.V.**	**Sample**	**Mean Rate (mA/min)**	**Std Dev**	**% C.V.**
Control 1	409	2.9	0.7	Control 1	439	4.5	1.0
Control 2	447	4.3	1.0	Control 2	480	4.8	1.0
Pool	439	3.9	0.9	Pool	470	4.9	1.0

All samples urine based: Control 1 = 150 ng/mL Oxazepam, Control 2 = 300 ng/mL Oxazepam: Pool = 1250 ng/mL lorazepam glucuronide

Results were within the manufacturer's specified acceptance criteria: within-run imprecision ≤2.0%, total imprecision ≤3.0%.

b. Linearity/assay reportable range:

Not applicable; this assay is intended for qualitative determinations.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Accelerated stability testing compared the recoveries of control materials and lorazepam glucuronide-spiked urine stored at 25 ºC and 4 ºC materials that were stored at -20 ºC since time zero. The sponsor states that the accelerated stability data supports a shelf-life claim of 18 months and that real-time stability studies are ongoing.

The DAT Multi-Drug calibrators (Negative, Low Urine, and High Urine) that are used in this assay were cleared under k944075, and k993954.

d. Detection limit:
The sponsor did not claim a detection limit.

e. Analytical specificity:
Cross-reactivity was tested by spiking negative urine (pooled) with various concentrations of cross-reactant and assaying these solutions with the benzodiazepine immunoassay. The lowest concentration that gives a **positive** result (where the change in absorbance per minute is greater than the cutoff calibrator) is reported below:

BNZG Cross-Reactivity

COMPOUND	Conc. (ug/mL)	COMPOUND	Conc. (ug/mL)
Oxazepam (cutoff)	0.2	Flurazepam	0.1
Alprazolam	0.2	Halazepam	0.1
a-hydroxy-Alprazolam	0.1	a-hydroxy-Alprazolam glucuronide	0.125
7-Aminoclonazepam	0.8	Lorazepam	0.4
7-Aminoflunitrazepam	0.5	Lorazepam glucuronide	1.0
7-Aminonitrazepam	0.75	Lormetazepam	0.2
Bromazepam	0.3	Medazepam	0.2
Chlordiazepoxide	0.3	Nitrazepam	0.3
Clobazam	0.5	Nordiazepam	0.05
Clonazepam	0.3	Oxazepam glucuronide	0.7
Delorazepam	0.1	Prazepam	0.2
Desalkylflurazepam	0.1	Temazepam	0.2
N-Desmethylflunitrazepam	0.3	Temazepam glucuronide	0.3
Diazepam	0.05	a-hydroxy-Triazolam	0.075
Flunitrazepam	0.2	Triazolam	0.05

No interference was seen when the following compounds were spiked into negative urine at the listed concentrations:

COMPOUND	Conc. (ug/mL)	COMPOUND	Conc. (ug/mL)
Acetaminophen	1000	Morphine	200
Acetylsalicylic acid	1000	Oxaprozin	10
Albuterol	1000	Pemoline	1000
d-Amphetamine	1000	Phencyclidine	1000
Caffeine	100	Promethazine	100
Codeine	1000	Propoxyphene	1000
Dextromethorphan	1000	Secobarbital	1000
Diphenhydramine	500	Sertaline	500
Doxepine	1	Tramadol	1000
Hydroxyzine	40	Trazodone	1000
Mesoridazine	1000	Trimipramine	100
Methadone	1000	Trimethoprim	1000
Metronidazole	1000	Zolpidem	100

Accurate qualitative results were obtained when negative calibrator, Multi-Drug Low and High calibrator, and lorazepam glucuronide matrices were spiked with the following: ascorbic acid (20 mg/dL), albumin (500 mg/dL), glucose (3 g/dL), hemoglobin (300 mg/dL), pH (3 and 9), and urea (6 g/dL).

It is possible that other substances and/or factors not listed above may interfere with the test and cause false positive or false negative results.

f. Assay cut-off:

The identified cutoff concentration of the assay is 200 ng/mL oxazepam. The sponsor determined how the device performs around the claimed cutoff concentration by testing 20 samples each of the oxazepam low control (150 ng/mL oxazepam), the oxazepam cutoff calibrator (200 ng/mL oxazepam), and oxazepam at 250 ng/mL on the Synchron CX and LX platforms. Results are shown in the table below:

BNZG Performance Around the Cutoff (200 ng/mL Oxazepam)

CX4 PRO – Cutoff Rate 416.9					**LX20 PRO** – Cutoff Rate 431.8			
Concent'n Oxazepam	Qualitative Result	Correct Calls	Mean Rate ± SD		Concent'n Oxazepam	Qualitative Result	Correct Calls	Mean Rate ± SD
150 ng/mL	Negative	20/20	404 ± 3.5		150 ng/mL	Negative	20/20	421 ± 3.0
200 ng/mL	Positive	17/20	419 ± 4.1		200 ng/mL	Positive	19/20	436 ± 3.0
250 ng/mL	Positive	20/20	431 ± 3.5		250 ng/mL	Positive	20/20	449 ± 2.0

2. Comparison studies:

a. Method comparison with predicate device:

Known Drug-of-Abuse urine samples were obtained from a clinical source and a set of presumed negative urine samples were obtained from in-house donors for a total of 158 samples. Samples were screened on Synchron systems to categorize the samples relative to the qualitative cutoff concentration. Sample rates below the cutoff rate were considered negative while rates above the cutoff rate were considered positive.

All 158 samples were sent to a reference laboratory for GC/MS screening for: lorazepam, oxazepam, α-OH-alprazolam, and α-OH-triazolam. If the initial GC/MS screen came back positive no further testing was performed; thus not all benzodiazepines and/or metabolites were tested for by GC/MS. If the initial GC/MS screen did not account for the Synchron positive result then the sample was screened for: diazepam, nordiazepam, temazepam, and Midazolam (33 samples). Samples that still were positive by Synchron but negative by the two GC/MS methods (10 samples) were analyzed by qualitative comprehensive GC/MS drug screens. *Any amount of benzodiazepine detected by GC/MS was considered positive in this*

concordance study.

Testing was designed so 25% of the samples were within ±10% of the Synchron rate response cutoff rather than within ±50% of the GC/MS cutoff concentration.

BNZG Concordance Summary: Synchron CX and LX Systems*

		Synchron BNZG		
		POS	**NEG**	Total
GC/MS	**POS**	93	2	95
	NEG	10	53	63
	Total	103	55	158

***Testing the samples on each system yielded the same results.

Overall agreement between the methods was 92%. Both false negatives contained a-hydroxy-alprazolam and one also contained lorazepam; their rates were 1.6% and 6.3% below the system rate cutoff. Ten samples were positive by the BNZG test and negative by GC/MS. Four of these false positives had borderline rates at 0.6%, 1.7%, 3.6%, and 5.2% above the system rate cutoff; they were not characterized by the qualitative drug screen. The other six false positives are detailed below:

Sample	**% Above Cutoff Rate**		**Compounds in Sample**
	CX	**LX**	
1	51%	49%	Bupropion and metabolites, Caffeine
2	17%	15%	Quetiapine metabolites, Caffeine
3	67%	65%	Metoclopramide, Fluoxetine and metabolite, Ticlopidine, Hydrozine metabolite, Caffeine
4	61%	59%	Quetiapine metabolites, Acetaminophen
5	69%	66%	Bupropion and metabolites, Citalopram, Topiramate, Acetaminophen, Nicotine metabolite
6	9.7%	5.1%	Nicotine metabolite

The study included an acceptable number of samples that had a rate response close to that of the cutoff values (± 10%). Performance of the assay around the cutoff compared to GC/MS is shown below:

Agreement of Methods Around the Assay Cutoff; Clinical Samples

	LX20 BNZG (n=36)				CX BNZG (n=35)			
		+10% C/O	**-10% C/O**				**+10% C/O**	**-10% C/O**
GC/MS	**POS**	12	1		**GC/MS**	**POS**	12	1
	NEG	6	17			**NEG**	6	18

b. Matrix comparison:

Not applicable; this device is only for urine samples.

3. Clinical studies:
   a. Clinical Sensitivity:
   Not applicable to this type of device.
   b. Clinical specificity:
   Not applicable to this type of device.
   c. Other clinical supportive data (when a. and b. are not applicable):

4. Clinical cut-off:
   Not applicable.
5. Expected values/Reference range:
   Not applicable.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.